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Contractor: BIONETICS RESEARCH LABORATORIES, INC.

Contract No.: DA 18-108-AMC-119(A)

SECOND QUARTERLY PROGRESS REPORT

Covering the Period

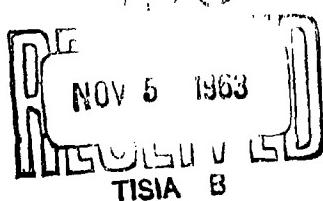
22 June 1963 - 22 September 1963

Title: New Methods Development for Irritant Screening

Prepared by

E. ROSS HART, Ph.D.

Date: 15 October 1963



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| Bionetics Research Laboratories, Inc. | Bionetics Research Laboratories, Inc.

| Falls Church, Va. | Falls Church, Va.

| NEW METHODS DEVELOPMENT FOR IRRITANT | NEW METHODS DEVELOPMENT FOR IRRITANT |

| 1. Irritation, | 1. Irritation, |

| skin and eye | skin and eye |

| SCREENING - E.Ross Hart | SCREENING - E.Ross Hart |

| Report No. 2, 22 September 63, 5 pp | Report No. 2, 22 September 63, 5 pp |

| Contract DA 18-108-AMC-119(A) | Contract DA 18-108-AMC-119(A) |

| 4C08-03-016-07 | 4C08-03-016-07 |

| During second quarter, assessment by | During second quarter, assessment by |

| current methods was emphasized. Screening | current methods was emphasized. Screening |

| completed on approximately 100 compounds. | completed on approximately 100 compounds. |

| Remainder of 100 compounds will be | Remainder of 100 compounds will be |

| completed by middle of third quarter. | completed by middle of third quarter. |

| Rat mast cells supply inadequate histamine | Rat mast cells supply inadequate histamine |

| for test of liberation by irritants. | for test of liberation by irritants. |

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INTRODUCTION

This second quarterly progress report presents a statement of work accomplished during the period from 22 June, 1963 to 22 September, 1963 by the contractor, Bionetics Research Laboratories, Inc., under Contract No. DA 18-108-AMC-119(A).

The report is divided into sections which correspond to the sections of the Statement of Work of the Contract. Thus, Section I (New Methods Development) discusses the progress which has been made in that phase of the work. Section II (Assessment of Irritant Potential) discusses the screening operations under the corresponding phase of that work.

It seems appropriate to point out here that, in accordance with plans developed in conference with the contract project officer, the screening work (Section II) has received the larger share of emphasis during the period herewith reported upon. This was done with the objective of achieving a high degree of efficiency of operation by utilizing a well-trained team full time. That this objective has been achieved will, it is believed, be clear from what follows in Section II. In future period the proportion of emphasis on the two phases of the contract will shift, for the obvious reason that the screening work is nearing completion.

SECTION I - New Methods Development

A. Work accomplished

Histamine liberation

As mentioned in the first progress report, the decision was made that the investigation of the histamine-liberating characteristics of irritants was to be explored first. It was further decided that the amounts of histamine present in tissues before and after exposure to irritants would be measured by a fluorometric technique, and that the first "tissue" examined would be mast cells isolated from rat peritoneal fluid. At present the fluorometric assay of histamine is proceeding smoothly and presents no difficulties. The method for collection of mast cells originally adopted (Uvnäs and Thon, Exptl. Cell Res., 18: 512, 1960 and 23: 45, 1961) presented difficulties in that it provides an inadequate number of mast cells. It has, therefore, been necessary to modify this procedure. That is, instead of withdrawing fluid from the peritoneal cavity of animals previously injected and sacrificed, we are now maintaining a number of animals with regular peritoneal injections and fluid withdrawals in order to produce more massive ascites and consequently larger numbers of mast cells. Preliminary evaluation of mast cells as a source shows that only a limited amount of histamine is released on incubation with irritants. Furthermore, it has thus far shown the differences between irritants to be disappointingly small. At present it is felt that a more adequate survey should be made of the various conditions of incubation of irritants with the cells before this technique is abandoned.

2.

Species Survey

A start has been made on the suggested survey of the eyes of a variety of species of animals for use in irritant evaluation. As mentioned in the previous quarterly report, this work has been deliberately deferred in order to allow the project personnel to achieve a high degree of familiarity with the currently used procedures. The survey is now being carried on at a rate that will not unduly interfere with the on going screening operations. The tentative findings, to date are these. After a brief trial, the mouse has been rejected as unsuitable due to his small size. Although some difficulties are encountered in proper restraint of these animals, the principal difficulty is that the mouse eye is so small that there is extreme difficulty in detecting the details of the irritant effect. Similarly a limited number of trials with dogs permits some tentative conclusions. However, these are subject to possible revision when larger numbers of animals and a greater variety of compounds have been tested. Essentially, the same spectrum of signs are produced in the dog as in the rabbit. However, all signs appear to be less intense and to last for a shorter period of time in the dog than in the rabbit. The tentative conclusions are that: (1) the dog is somewhat less sensitive to eye irritation; (2) that the usual dark-colored iris in the dog makes examination somewhat more difficult, and (3) that the combination of blepharospasm and extension of the nictitating membrane which occurs in some dogs in the presence of irritation makes it difficult or occasionally even impossible to observe the iris and cornea.

B. Plans for subsequent period:

It has been decided to explore other tissues as sources of histamine in the hope that they may prove more sensitive to histamine liberation by irritants than appears to be the case with rat peritoneal mast cells. The next tissue to be explored is the guinea pig lung. The species survey will, in the very near future, be extended to include cats and certain species of monkeys. With respect to species of monkeys, effort will be made to obtain species with relatively light-colored irises for reasons previously mentioned. At a recent conference with the Contract Project Officer it was decided to initiate exploratory studies of ciliary activity and bronchial muscle (tracheal chain). Steps have been taken to acquire the necessary animals and equipment to follow the procedures described in the proposal.

PART II - Assessment of Irritant Potential

A. Compounds received:

Received 7/24/63

CS 9784	CS 39230	CS 40844
CS 23412	CS 39232	CS 40849
CS 24865	CS 39233	CS 40850
CS 24801	CS 39239	CS 40877
CS 28938	CS 39241	CS 41455
CS 31676	CS 39242	CS 41458
CS 31677	CS 40834	CS 41460
CS 31688	CS 40840	CS 41462
CS 39228	CS 40841	CS 41467
CS 39229	CS 40843	CS 41468

CS 41469	CS 41623	CS 42811
CS 41470	CS 42213	CS 42812
CS 41543	CS 42214	CS 42818
CS 41576	CS 42216	CS 42819
CS 41577	CS 42218	CS 42820
CS 41591	CS 42737	CS 42821
CS 41592	CS 42739	CS 42822
CS 41593	CS 42740	CS 42824

Received 8/22/63

CS 27644	CS 42056	CS 42990
CS 39651	CS 42057	CS 42997
CS 39654	CS 42064	CS 42998
CS 39666	CS 42066	CS 43000
CS 41722	CS 42069	CS 43001
CS 41723	CS 42224	CS 43054
CS 41724	CS 42354	CS 43157
CS 41725	CS 42355	CS 43166
CS 41726	CS 42356	CS 43168
CS 41727	CS 42982	CS 43169
CS 41763	CS 42983	CS 43175
CS 41864	CS 42984	CS 43176
CS 42039	CS 42985	CS 43178
CS 42049	CS 42986	CS 43179
CS 42054	CS 42987	CS 43180
CS 42055		

B. Work completed:

Assessment has now been completed on the following compounds which were incomplete at the time of the first report.

CS 15414
 CS 15442
 CS 18639
 CS 20409
 CS 23654

Additional compounds on which complete assessment has been accomplished:

CS 9784	CS 36828	CS 39239
EA 118-055-5	CS 36870	CS 39241
EA 119-400-1	CS 36871	CS 39886
CS 19843	CS 37267	CS 39902
CS 23412	CS 37268	CS 39903
CS 24865	CS 37269	CS 40320
CS 25299	CS 37270	CS 40323
CS 28401	CS 38355	CS 40324
CS 31627	CS 38356	CS 40325
CS 31638	CS 38730	CS 40331
CS 31641	CS 38731	CS 40332
CS 31642	CS 38739	CS 40669
CS 31677	CS 39228	CS 40677
CS 34927	CS 39233	CS 40679
CS 34928		

CS 40683	CS 41470	CS 42213
CS 40686	CS 41577	CS 42216
CS 40781	CS 41591	CS 42739
CS 40785	CS 41593	CS 42740
CS 40800	CS 41623	CS 42818
CS 40801	CS 41722	CS 42819
CS 40804	CS 41725	CS 42820
CS 40805	CS 41726	CS 42822
CS 40806	CS 41763	CS 42985
CS 40840	CS 41864	CS 42986
CS 40844	CS 42049	CS 43001
CS 41455	CS 42055	CS 43166
CS 41467	CS 42056	CS 43168
CS 41468	CS 42057	CS 43176
CS 41469		

Attention is called to an error which has been discovered in the first report. Complete assessment was reported for CS 17593. This should read CS 17953

Compounds on which assessment is incomplete (evaluated for skin irritation only):

CS 31626	CS 42039
CS 39654	CS 42355
CS 40678	CS 42984
CS 40685	CS 42987
CS 40797	CS 42990
CS 40843	CS 42997
CS 41723	CS 43054

Compounds on which assessment is incomplete (evaluated for eye irritation only):

CS 28938	CS 41592
CS 39242	CS 42214
CS 40834	CS 42218
CS 40841	CS 42747
CS 40849	CS 42811
CS 40850	CS 42821
CS 41460	CS 42824
CS 41462	CS 42998
CS 41543	CS 43000
CS 41576	CS 43169

As provided in the contract, technical reports on this phase of the work have been submitted monthly to the Contract Project Officer.

C. Discussion:

By devoting the full time effort of a trained team to this phase of the work the equivalent of complete assessment of approximately 100 compounds has been accomplished (83 complete; 16 eye only; 24 skin only). This fulfills the prediction made in the first report. In fact schedules are now in operation which accomplish complete evaluation of approximately 15 compounds per week.

D. Work planned

On the basis of schedules which have been in operation during the last portions of this report period; we confidently predict complete assessment of the planned 200 compounds by approximately the middle of the third quarter of the contract.

SUMMARY

During the second quarter of the contract emphasis has been placed on assessment of compounds by current methods. Histamine liberation from rat mast cells has been found to be small and other tissues are being considered. New directions for methods development are being explored.

Assessment by current methods has been completed on approximately 100 compounds (83 eye and skin; 16 eye only; 24 skin only). Schedules now in operation will permit completion of assessment of the remaining compounds by the middle of the third quarter.